REVIEW ARTICLE

Plasma Cell Myeloma—New Biological Insights and Advances in Therapy

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PLASMA CELL myeloma has been the prototype of a monoclonal tumor cell proliferation that reveals a monoclonal protein in the serum or urine in more than 90% of patients. The disease spans a spectrum of clinical entities, including localized and disseminated, as well as indolent and aggressive forms.[1-3] Patients typically suffer from painful fractures, anemia or renal failure, and from recurrent infections associated with immunodeficiency. With a median age of 60 years, current treatments have been palliative rather than curative.[5] Thus complete remissions from standard alkylating agent-glucocorticoid programs have been achieved infrequently, and all patients succumb after a median of about 3 years, even though about 10% of current patients will survive 10 years.[6]

During the past 5 years, new insights have been gained of the cellular and molecular biology of myeloma, so that the previous notion of a terminally differentiated B-cell malignancy is giving way to the provocative concept of an early hematopoietic stem-cell disorder manifesting itself mainly at the mature stage of B-cell lineage. Therapeutically the growth-controlling effects of alpha-interferon[7] and the feasibility of hematopoietic stem-cell–supported high-dose cytotoxic regimens[8] have raised hope for a fundamental change in prognosis that had been stagnant since the introduction of melphalan and prednisone some 25 years ago.[9-11] This review summarizes data that myeloma may be an early stem-cell disorder; describes features that predict clinical outcome; and reviews marrow-ablative programs for patients with newly diagnosed and refractory disease. Readers with interest in traditional staging and treatments are referred to other recent reviews.[12-15]

MYELOMA BIOLOGY

Genotypic Studies

Because of the low fraction of cells in DNA synthesis, few mitoses are usually available to define karyotype abnormalities.[16,17] Unlike results in leukemia and lymphoma, disease-specific anomalies have not yet been identified; the presence of complex numeric and structural rearrangements suggests a long disease course with many acquired genetic changes. Analysis of nuclear DNA content by flow cytometry (that does not depend on mitoses) has demonstrated aneuploidy in 80% of patients; hyperdiploidy was most frequent (70% of total, with a DNA excess of 10% to 20%); diploidy was present in 20%; and hypodiploidy in about 10%.[18-20] Also confirmed by cytogenetic studies, DNA-hypodiploidy was associated frequently with only light-chain production and with resistance to standard therapies. (Table 1).[17,21] Chromosomal translocations, as in non-Hodgkin's lymphoma, were found in about 10% of cases, and t(8;14) abnormalities were associated with an IgA isotype.[17] Deletions of the long arm of chromosome 6 have recently been linked to lytic bone disease.[22] An adriamycin-resistant myeloma cell line (8226/DOX) displaying the multidrug resistance (MDR) phenotype has a deletion of the long arm of chromosome 7,[23] which is the site of the p-glycoprotein gene.[24]

While the acquisition of new DNA stemlines on flow cytometry is rare,[25] this technique is insensitive for the detection of structural aberrations or small numeric chromosomal abnormalities.[26] Indeed, the typical complexity of plasma cell karyotype suggests that clonal evolution must be common. With the prospect of better in vitro culture methods, the authors expect that primary and evolutionary karyotypic anomalies will be identified regularly in myeloma progenitor cells. Such information may help define discrete disease entities and direct research into the molecular mechanisms of abnormal growth and differentiation.

Cell Kinetics and In Vitro Growth of Tumor Cells

Laboratory methods for cytokinetic studies have been refined, so that autoradiography with tritiated thymidine has been replaced by the more convenient bromodeoxyuridine (BUdR) immunofluorescence technique.[27,28] As expected in a tumor composed mainly of terminally differentiated B cells (ie, plasma cells), the labeling index after in vitro pulse exposure is usually low, with average values of 1% at diagnosis and higher values at relapse.[29] Both autoradiographic and BUdR techniques have identified even lower labeling indices in patients with monoclonal gammopathy of unknown significance (MGUS) and indolent myeloma.[30-32] A similar discrimination also resulted from the study of B lymphocytes in peripheral blood.[32,33] In patients with symptomatic myeloma, higher values of plasma-cell labeling index were associated with shorter survival time, independent of tumor mass.[34-36]

Relative to values at diagnosis, tumor cell-growth fraction (determined from tritiated thymidine administration in vivo) was much higher during relapse,[37] when in vitro colony growth is also more successful. The recent availability of antibodies that distinguish different DNA precursor molecules (BUdR and IUdR) permits a more expedient immunofluorescence-based analysis by flow cytometry of cell-cycle time parameters and growth fraction (J Gray, personal communication, June 1988). Several studies are currently in progress in myeloma and other malignancies to define the value of Ki-67 monoclonal antibody (MoAb) as a means of assessing the fraction of cells capable of cell proliferation.[38,39]

Consistent with the low proliferative activity of typical plasma-cell myeloma, attempts at establishing long-term

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Table 1. Clinically Relevant Chromosomal Anomalies

Cytogenetic Abnormality	% Incidence	Associations	Reference
Hypodiploidy	10	Only Bence Jones protein	17
		Primary drug resistance	21
t (8;14) (q24;q32)	<5	IgA	17
6q−	15	Lytic bone disease	22
7q−	?	Drug resistance, site of mdr gene	23
Trisomy 11	10	Decreased p21 (H-ras) levels	111

cultures have met with limited success, so that only about a dozen human myeloma cell lines are now available, often with unusual phenotypic features typical of earlier stages of maturation.[40] Similar problems were met in sustaining short-term cultures for the study of in vitro drug sensitivity.[41,42] Systematic laboratory efforts by Durie et al (BGM Durie, personal communication, October 1987) have since led to the recognition that monocytes and CD4-positive T cells provide important stimuli for in vitro growth of plasma cells and their precursors, while CD8-positive suppressor T cells seemed to inhibit growth. Employing a double-layer agar technique with irradiated HL-60 feeder cells, Millar et al.[43] reported successful myeloma colony growth in almost 90% of patients.

Several cytokines are involved in the proliferation and differentiation of normal murine and human B cells.[44,45] After B-cell activation by interleukin-4 (IL-4)[46,47] and expansion by interleukin-5 (IL-5),[48] B-cell stimulatory factor 2 (BSF-2) induces the final maturation of B cells into immunoglobulin-secreting plasma cells.[49] Based on studies of DNA sequence homology, BSF-2, interferon-beta-2, and hybridoma-plasmacytoma growth factor represent the same cytokine that is now termed interleukin-6 (IL-6).[50,51] Produced by monocytes, fibroblasts, and T-cell lines, the dominant action of IL-6 is the differentiation of activated B cells, although effects on normal hematopoietic stem cells (in concert with IL-3) have also been reported.[52,53] IL-6 probably accounts for the promotion of human myeloma growth by conditioned medium from adherent spleen cells when Balb/c mice were primed by intraperitoneal injections of pristane or mineral oil.[41] The recent demonstration that fresh myeloma cells produced IL-6 constitutively and expressed IL-6 receptor (IL-6R), with stimulation of DNA synthesis by exogenous IL-6 in some cases has suggested that IL-6 may function as an autocrine growth factor.[54] This observation has been questioned by Klein et al,[55] who noted that IL-6 activity resided entirely in bone marrow-adherent cells and favored a paracrine growth mechanism. These authors had previously reported an autocrine function for BCGF II (IL-5) in human RPMI 8226 cells.[56] Investigating the molecular basis of growth factor and receptor gene activation should clarify the pivotal mechanisms involved in the abnormal proliferation and maturation of plasma-cell myeloma.

Tumor Phenotype

While an individual patient has fairly uniform plasma cell morphology, there is moderate heterogeneity among different patients. The Mayo Clinic group has distinguished between plasmacytic, lymphoid, and plasmablastic variants with progressively increasing labeling index and adverse prognosis.[30,57] As a tumor perceived to be composed of terminally differentiated B cells with commitment to immunoglobulin production and secretion, plasma-cell myeloma is composed mainly of cells that contain monoclonal cytoplasmic immunoglobulin.[58] Monoclonal cIg expression by diploid cells in about 20% of patients with DNA-aneuploidy indicated the presence of DNA-biclonality that was also associated with resistance to chemotherapy.[21] Surprising was the coexpression of both kappa and lambda light chains in the same DNA-aneuploid tumor cells in about 15% of patients, usually with IgG lambda isotype.[58] The molecular basis of these aberrations from the current dogma of exclusive kappa or lambda light-chain expression and their association with gamma heavy chains remains to be clarified.[59,60]

Consistent with their major commitment to protein secretion, myeloma cells contain high levels of RNA, an average six times more than unstimulated lymphocytes.[61,62] Exploiting the metachromatic properties of acridine orange, cellular DNA and RNA content can be measured simultaneously by two-parameter flow cytometry quantitating green (double-stranded DNA) and red fluorescence (single-stranded RNA).[63] The detection of aneuploidy also in blood provided direct evidence for circulating tumor cells,[25] long suspected on the basis of monoclonal idiotype-concordant blood lymphocytes[64-66] capable of plasma-cell differentiation in vitro.[67] Thus disease progression and spread may occur by the hematogenous route, with specific tumor localization enforced by marrow-derived monocytes and T cells providing growth-stimulating factors.[47-49,55]

In addition to facilitating the quantitation of marrow plasmacytosis, flow cytometric studies of nucleic acids have provided useful prognostic information.[68] Thus no patient with DNA-hypodiploidy has ever responded to standard melphalan-prednisone or VAD; and higher response rates have been observed with increasing cellular RNA content. As with nuclear DNA, plasma-cell RNA index remained stable throughout a patient's disease and declined only rarely with relapse. Therefore the undoubted clonal evolution that produces drug resistance was not reflected in changes of cellular DNA and RNA content. Occasional mixed clinical responses to therapy could be traced to different DNA stemlines present in different disease sites.

While the majority of tumor cells have plasma-cell features, self-renewal must be sustained by stem cells earlier in the maturation sequence.[67,69,70] Several groups have searched for the expression of early B, T and myelomonocytic markers.[71,72] In almost 50% of patients, the pre-B antigen common acute lymphoblastic leukemia antigen (CALLA) was

Table 2. Differentiation and Lineage Infidelity

Phenotype	% Incidence	Comment	Reference
Calla+	50	Coexpression with cIg^+ and good prognosis	72
		Poor prognosis	73
Myelomonocytic	13	Poor prognosis when two myeloid markers present	74
T-antigen	?	Coexpression with cIg^+ and myeloid markers	74
TCR-gamma rearranged	40	No clinical correlation	102
LDH	50	High levels in terminal disease phase with lymphomalike features and poor prognosis	114

expressed in a large fraction of tumor cells (Table 2).[72] The coexpression of CALLA and monoclonal cIg by the same aneuploid tumor cells unmasked a novel tumor-cell phenotype without known counterpart in normal B-cell differentiation (Fig 1). Such differentiation infidelity was also shared by the mature plasma-cell antigen R1-3, which was jointly expressed with CALLA in about 40% of patients. The consistent presence, in all cases of DNA-aneuploid myeloma, of CALLA-positive cells with diploid DNA content (but without cIg expression) raised the possibility that diploid precursor cells generated the aneuploid plasma cells. Subpopulations of cells with $CALLA^+/cIg^-$, $CALLA^+/cIg^+$, and $CALLA^-/cIg^+$ may represent different maturation stages of plasma-cell myeloma. The authors have observed a more favorable clinical course in patients with CALLA expression, while others have concluded that this phenotype was harmful.[73]

Myelomonocytic antigen expression was reported recently in 13% of patients with myeloma (Table 2).[74,75] Those with dual myeloid antigen expression (M5 and MY7) also showed T- and early B-cell features, high labeling index, and a poor prognosis. As expected from frequent elevations of serum beta-2-microglobulin (B2M) level, most patients showed tumor cell-surface expression of B2M.[72] Both surface expression and serum levels of B2M can be augmented after treatment with interferon-alpha, although correlations with clinical response have not been detected (Epstein J, Barlogie B, Alexanian R, unpublished observations, June 1988).

Thus myeloma is a disease in which the majority of tumor cells are aneuploid with a differentiated B-cell phenotype but with subpopulations that also express early B, possibly T, and even myelomonocytic features. The additional presence of diploid cells with an immature phenotype suggests that aneuploid myeloma originates from a stem cell with a different (ie, diploid) DNA complement.[72] While differentiation and lineage infidelities may reflect merely malignancy-associated aberrations, they may indicate alternatively that transformation involved a pluripotent stem cell with a flexible pattern of gene expression. More specifically, the asynchrony of antigen expression in myeloma may reflect

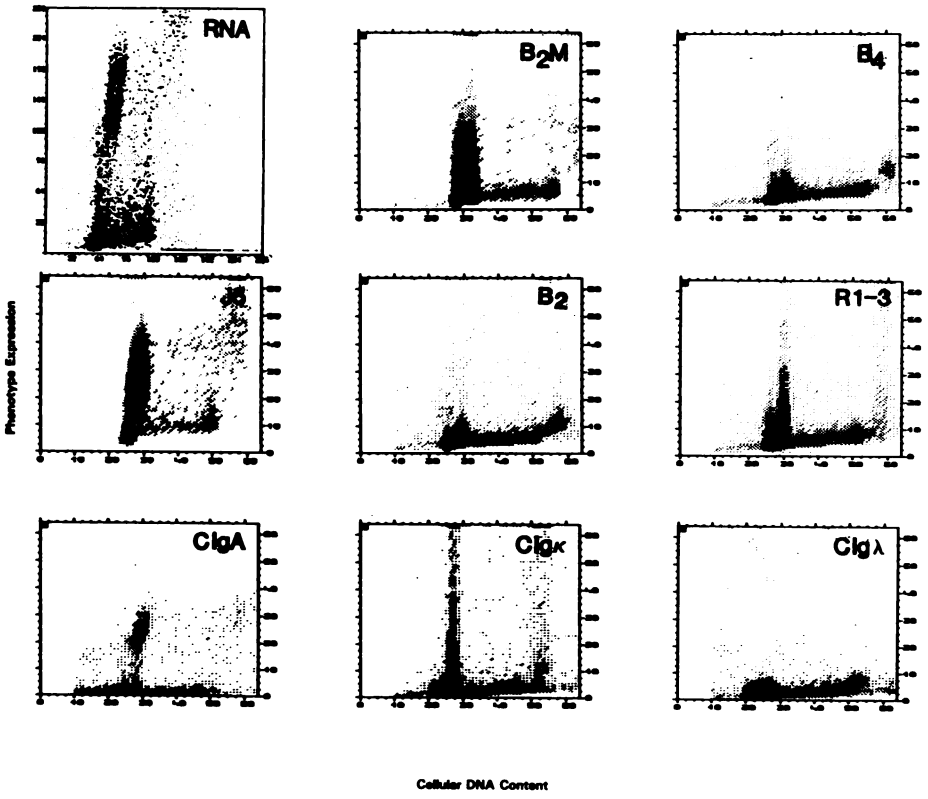

Fig 1. Phenotypic heterogeneity in a patient with hyperdiploid multiple myeloma. Hyperdiploid cells have high RNA content; react with anti-B_2M, J5, and R1-3 and show monoclonal cIgA kappa expression. Diploid cells have low RNA content without monoclonal cIg reactivity but express B_2M and CALLA. Abscissa, DNA content; ordinate, fluorescence intensity of cellular feature.

Table 3. Factors Produced by Myeloma Cells

Factor	Comment	Reference
IL-1 beta	OAF, chromosome 2q; autocrine growth factor?	78 79
TNF-beta	OAF, chromosome 6p21	77
TNF-alpha	Autocrine growth factor in CLL, HCL; not evaluated in myeloma; on chromosome 6p21	80
BCGFII (IL-5)	Myeloma cell line (RPMI 8226)-autocrine growth factor (?); chromosome 5q23.3-q32	56
IL-6	Myeloma cells and marrow-adherent cells	54 55
B_2M	Increased in most patients; poor prognosis with high serum levels; chromosome 15q21-q22	138-141

abortive differentiaton during an ordered rather than stochastic hematopoietic development with sequential commitment to megakaryocytic, erythroid, myeloid, and, finally, B-cell lineage.[76] The demonstration of erythroid or megakaryocytic antigens on myeloma cells would support such a model (Epstein J, Barlogie B, unpublished observations, December 1988).

Consistent with the variety of disease features, myeloma cells produce many cytokines in addition to immunoglobulins. Osteoclast-activating factors have been identified as TNF-beta (lymphotoxin) and IL-1-beta (Table 3).[77-79] While usually undetected in mature B cells, the continued expression of TNF-beta and IL-1-beta by myeloma cells[78] suggests a role for those proteins in abnormal growth and differentiation, also invoked for IL-5[56] and IL-6.[54] Indeed, TNF can act as a tumor growth factor and can promote cell proliferation in two other related B-cell malignancies, namely hairy cell and chronic lymphocytic leukemia.[80] Receptors for IL-5 (BCGF II) and IL-6 on myeloma cells may provide targets for therapy with immunotoxins or radioisotopes. Myeloma cells also express receptors for vitamin D_3, glucocorticoids, and sex hormones (estrogens and progestins; Table 4).[81-84] Future studies should clarify the biological functions and implications of these receptors in the different B-cell tumors. In view of the clinical use of glucocorticoids[85] and alpha-interferon,[86] correlations of response with receptor expression are important.[81,87]

Immunodeficiency mainly of B-cell, and less profoundly and frequently of T-cell, type is responsible for recurrent infections, usually of bacterial origin.[88,89] In murine plasmacytoma, B-cell suppression has been traced to a plasma-cell factor that causes monocytes and macrophages to produce a suppressor of normal B-cell proliferation[90]; a similar factor has not yet been identified in humans. An alternative mechanism concerns the suppression of normal B cells by immunoglobulin-binding factor (IgBF), which may be identical to the Ig-Fc receptor shed by T cells.[91] In the murine MOPC-315 model, IgA-binding factor produced by IgA-induced T cells reacted with surface IgA on MOPC-315 cells; this resulted in the inhibition of both tumor growth and Ig production by the suppression of myc and Ig gene transcription, respectively.[92]

The resistance to cytotoxic drugs poses a major obstacle to the successful management of myeloma and other neoplastic diseases. Refractoriness to multiple antitumor agents after exposure to one drug is referred to as MDR, a feature that eventually becomes dominant in cultured tumor cells.[23,93] The MDR phenotype has been linked to the presence of a p-glycoprotein (p170) that functions as an efflux pump for certain anticancer agents, such as anthracyclines and vinca alkaloids.[94,95] Using the MoAb, C219, which recognizes the cytoplasmic domain of p170,[96] high levels of MDR expression have been observed in myeloma cells and have been linked to clinical resistance to the VAD regimen.[97] Such resistance has been overcome with the calcium channel blocker, verapamil,[98] presumably by retaining higher cellular levels of adriamycin.[99] The frequency and degree to which VAD resistance can be overcome with verapamil is now under study.[100]

Molecular Genetics

Studies of immunoglobulin gene rearrangement (as the molecular hallmark of a B-cell tumor) may explain the phenotypic heterogeneity and differentiation infidelity of myeloma.[53] As expected, J_H rearrangement could be detected in proportion to the degree of marrow plasmacytosis. Multi-

Table 4. Receptors Expressed on Myeloma Cells

Receptor for	Comment	Reference
BCGFII (IL-5)	Cell lines, autocrine mechanism (?)	56
IL-6	Cell lines and fresh samples; mediates autocrine or paracrine mechanism	54
Interferon-alpha	Active against low-mass disease; chromosome 21q21	7 86
Glucocorticoid	Cell lines and fresh samples; correlation with clinical response to glucocorticoids not yet established; chromosome 5q11-q13	81 85
Estrogen, progesteron	Biological effects (?); chromosome 6q24-q27 (ER) and 11q13 (PR)	83 84
Vit D3	Mediates inhibition of proliferation and induction of new phenotypes by Vit D3	82

ple rearranged bands were noted in only 5% of patients, indicating that clonal evolution was reflected rarely at the immunoglobulin gene level (Selvanayagam P, unpublished observations, September 1988). The concurrent rearrangement of Cu and J_H genes in some patients resulted either from different tumor clones in different stages of B-cell maturation or from the coexpression of normally unassociated phenotypes. The authors' finding of cIg kappa coexpression in IgG lambda myeloma was confirmed at the molecular level, with C_k rearrangement present in some patients with lambda light-chain production.

Since early stem cells may be the source of myeloma, analysis was also carried out of T-cell receptor genes, which are part of the immunoglobulin supergene family. Surprisingly, 35% of patients showed TcR-gamma rearrangement, while TcR-beta and TcR-alpha genes were not affected.[102] Identical frequencies of rearranged bands with TcR-gamma and J_H probes supported the neoplastic origin of TcR-gamma rearrangement.

In a survey of HLA-associated gene expression in human B-cell malignancies, invariant chain messenger RNA (In-mRNA) as well as HLA DR-alpha and HLA DR-beta-chain mRNA were expressed in all patients with either acute or chronic lymphocytic leukemia.[103] In contrast, only three of 15 patients with myeloma demonstrated In-mRNA expression. Thus the In gene was expressed in neoplastic cells early in the maturation sequence, concurrent with the activation of other genes encoding histocompatibility class II antigens, and preceded the activation of immunoglobulin genes.[104]

While advances in chromosomal banding and molecular techniques have clarified the role of cellular oncogenes in some B-cell lymphomas and leukemias, the difficulty in obtaining adequate metaphase chromosomes and the dearth of specific karyotypic aberrations have hindered similar progress in plasma cell myeloma. With the presumption of pre–B-cell involvement in human myeloma[72] and the regular *c-myc* gene deregulation in murine plasmacytoma,[105,106] *c-myc* and other oncogenes implicated in malignant lymphoma have been studied also in human myeloma (Table 5). High levels of *c-myc* RNA expression were observed in 25% of patients, without apparent abnormalities of myc RNA transcript size.[107] As with t(8;14) chromosomal translocations, myc gene activation was found more commonly in patients with IgA isotype but resulted only rarely from DNA rearrangement and was not associated with DNA amplification.[108] Analogous to recent observations in Burkitt's lymphoma, Meltzer et al[109] reported mutations in the 3′ region of the first *c-myc* exon (Pvu II and Alu I endonuclease sites) in ten of 16 human myeloma samples and cell lines; we have not confirmed this observation. None of 60 patients with myeloma showed rearrangement of *bcl*-2 (commonly involved in follicular lymphoma), consistent with the rarity of t(14;18) translocation in myeloma. Five of 120 patients had rearrangement of *bcl*-1 but without corresponding mRNA elevation.[110]

Table 5. Oncogenes in Plasma-Cell Myeloma

Abnormality	c-myc	L-myc	bcl-1	bcl-2	c-H-ras
Rearrangement	4/120	0/22	5/120	0/60	0/40
Amplification	0/120	0/22	0/120	0/60	0/40
Deletion	0/120	2/22	0/120	0/60	ND
High mRNA expression	9/37	ND	0/40	0/40	ND
High protein expression	ND	ND	ND		17/23

ND, not done.

Careful scrutiny of chromosomal breakpoints disclosed frequent involvement of the short arm of chromosomes 1 and 11,[17] where Kirsten-ras and Harvey-ras genes are located. Using pan-ras and H-ras antibodies with flow cytometry, 17 of 23 patients with active myeloma had high p21 expression.[111] An inverse relationship between p21 levels and trisomy 11 suggested that suppressor genes were present on chromosome 11.[112] The frequent elevation of p21 protein indicated that H-ras oncogene is involved in the pathophysiology of myeloma, as supported further by the shorter survival time of patients with high p21 levels.[111] The mechanism of H-ras gene activation is currently under scrutiny, especially in regard to gene mutation as the commonest mechanism of ras gene activation in other human tumors.

Biology Summary and Future Projections

Cytogenetic and molecular data indicate the presence in myeloma of DNA rearrangements similar to those observed in malignant lymphoma. Such chromosomal translocations presumably result from recombination errors during immunoglobulin gene rearrangement (ie, B-cell commitment) with activation of juxtaposed oncogenes.[113] Phenotypic evidence of differentiation and even lineage infidelity, with coexpression on terminally differentiated B cells of early B,[72] myeloid,[74] and T-cell markers[74] may reflect abortive differentiation in the process of an ordered sequential rather than a stochastic lineage commitment, thus placing the oncogenic events in myeloma possibly to an early stage in hematopoiesis.[76]

Disease evolution has not been studied systematically. Preliminary data suggest disease dedifferentiation occasionally with cessation of myeloma protein secretion and production instead of high levels of serum lactic dehydrogenase (LDH) during the terminal phase of disease transformation.[114] Similarly, the development of acute myeloid leukemia in up to 25% of patients 10 years after diagnosis of multiple myeloma has occasionally been interpreted as a natural disease evolution rather than as a treatment-induced secondary malignancy,[115] thus supporting the notion of myeloma as a tumor arising early during hematopoietic differentiation (Fig 2).

Sequential investigations during the disease course of individual patients are likely to reveal a progressive expansion of myeloma progenitor cells with higher proliferative activity and earlier phenotypic characteristics. Such information can then be used to probe for the presence of such features in rare cells earlier in the disease course.

Studies of human plasma-cell lines suggest that BCGF II (IL-5) may be an autocrine growth factor in some patients with myeloma.[56] Receptors for IL-6 (BSF-2) may mediate the biological activity of IL-6 produced mainly by bone marrow-derived monocytes (paracrine mechanism)[55] and/or by the tumor cells themselves (autocrine mechanism).[54] In view of the three-signal model advanced by Kishimoto[44,45] for

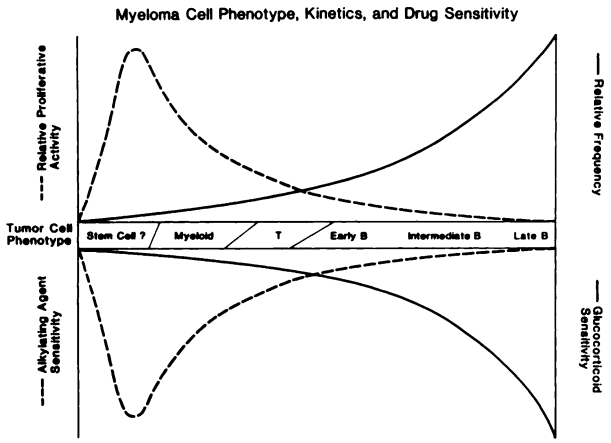

Fig 2. Model of myeloma ontogeny. On the basis of available phenotype and molecular data (see text), a DNA-diploid myeloma stem cell early in hematopoiesis is postulated with sequential abortive commitment to megakaryopoiesis, erythropoiesis, granulopoiesis, and, finally, B-cell lineage. Terminally differentiated B cells (plasma cells) represent the dominant tumor phenotype, usually with DNA-aneuploidy and frequently asynchronous expression of earlier stages of differentiation. Proliferation is highest at an early commitment stage with greater sensitivity to melphalan, whereas plasma cells (producing a variety of cytokines) are kinetically inactive and highly sensitive to glucocorticoids.

the activation, proliferation, and differentiation of normal B cells by IL-4, IL-5 and IL-6, these lymphokines probably play an important role in sustaining the growth and differentiaton anomalies of human myeloma. For example, the production by tumor cells of IL-1,[78,79] TNF,[77,80] and IL-4 or IL-5[56] may sustain both an autocrine growth-stimulatory loop and IL-6 production by bone marrow monocytes,[55] promoting the differentiation of earlier B cells into plasma cells. Abnormal differentiation with lineage infidelity (presence of myeloid and T-cell markers on plasma cells) suggests that receptors for B-cell growth and differentiation signals may also be expressed aberrantly. Comprehensive studies, at the single-cell level, of cytokine production by tumor cells, lymphokine receptor expression, proliferative activity, hematopoietic lineage, and phenotype stage are required to clarify these questions. Multiparameter flow cytometry[81,87] and image analysis, together with in situ hybridization, are suitable research tools for such investigations.

The key trigger to self-sustained and ultimately relentlessly progressive plasma-cell accumulation is likely to result from the abnormal expression of normal cellular genes by a variety of possible mechanisms, such as gene rearrangement, mutation, and/or lack of suppressor gene activity due to deletion of critical "antioncogenes."[112] High myc expression, usually without DNA rearrangement, in about one quarter of patients suggests that point mutations may be the major cause, resulting in impaired nuclear protein binding to myc DNA sequences and consequently uncontrolled growth, as in Burkitt's lymphoma.[116] Mutational activation of H-ras unopposed by controlling sequences on chromosome 11 may be an additional or separate mechanism of abnormal growth.

ETIOLOGY

In the mouse, an oil granuloma induces a preneoplastic chronic inflammation that progresses to plasmacytoma with myc gene deregulation[106]; in contrast, the etiology of human myeloma remains elusive. Several oncogenes (*c-myc* and H-ras) appear to be involved, but their link to the initiation and progression of myeloma is unclear. Radiation exposure, long known to induce myeloid leukemias and certain solid tumors, has sometimes led to myeloma 15 to 20 years later[117-121] Other epidemiologic surveys have demonstrated an association with benzene[122] and asbestos,[123] as well as with certain agricultural occupations.[124,125] Severe immunosuppression with organ transplantation, while inducing B-cell lymphoma, has rarely led to plasma-cell myeloma.[126] Similarly, while B-cell lymphomas have been recognized as a manifestation of the acquired immunodeficiency syndrome (AIDS), associations between human immunodeficiency virus (HIV) infection and myeloma remain anecdotal.[127,128] Only about 10% of persons with benign monoclonal gammopathy (one of the few benign disorders with DNA-aneuploidy of plasma cells[25]) develop myeloma after at least 10 years of follow up.[2] The persistence of a low-level monoclonal protein after marrow-ablative chemoradiotherapy for myeloma in remission suggests that a benign gammopathy often precedes myeloma and is resistant to supralethal doses of therapy because of the low proliferative activity of BMG-associated plasma cells.[129]

The presence of tumor cells in peripheral blood is relevant to an understanding of disease progression and has been suggested by several studies, including (1) isotype-concordant surface immunoglobulin expression by idiotypic lymphocytes[64,65]; (2) differentiation of CALLA-bearing peripheral B cells to monotypic plasma cells in vitro[63]; (3) circulating DNA-aneuploid cells[20]; and (4) presence of immunoglobulin gene rearrangement in blood.[66] Werne et al[130] examined the ratio of kappa- and lambda-positive lymphocytes in blood and noted the phenomenon of light-chain isotype suppression in stable phases of disease, contrasting with isotype predominance during disease progression.

DIAGNOSIS AND STAGING

Solitary and Indolent Myeloma

Solitary plasmacytoma of bone or soft tissue,[131] indolent or smoldering myeloma,[3,4,132] and symptomatic generalized myeloma[133] represent distinct clinical phases in the spectrum of plasma-cell myeloma (Table 6). The occurrence by chance of a painful pathologic fracture from a single tumor mass apparently allows the recognition of this disease about 5 years earlier than otherwise possible. Provided strict criteria for staging are applied, local radiotherapy appears to be curative in about one half of the patients. Staging criteria include no signs elsewhere of monoclonal plasmacytosis (best by DNA-cIg flow cytometry), lack of other bone lesions or abnormalities on sensitive radiographic examinations (computerized axial tomography [CAT] and nuclear magnetic resonance), and preservation of normal immunoglobulin

levels. Less rigorous criteria probably explain the divergent conclusions reached in regard to the natural history of this entity. Following radiotherapy, abnormal globulin levels are usually reduced markedly, indicating that the localized disease was encompassed by the radiation field. However, low monoclonal protein levels may persist indefinitely in some patients, indicating residual plasma cells that remain dormant in a manner analogous to BMG. Some localized plasmacytomas are radioresistant (eg, those with DNA-hypodiploidy or with low RNA content), portending resistance to later chemotherapy and a poor prognosis.[68] The curability of localized plasmacytoma in a substantial proportion of patients indicates that myeloma arises from a single focus; the persistence of monoclonal gammopathy without later evolution of generalized disease suggests that BMG is a preneoplastic entity, requiring additional factors to progress to myeloma. A similar explanation may pertain to the persistence of low levels of abnormal globulin after high-dose consolidation therapy for responsive multiple myeloma.[129]

When a solitary lesion is not associated with bone destruction (ie, perhaps because IL-1 or TNF-beta production is low), generalized myeloma may develop and progress slowly without symptoms. Only a chance electrophoresis leads to the diagnosis of such indolent or smoldering disease. Similar to patients with early phases of CLL or nodular lymphoma, chemotherapy is unnecessary until morbidity occurs or becomes imminent (ie, myeloma protein >5 g/dL or new bone lesions). The similar prognosis of patients treated many months after diagnosis to that of the usual patient treated promptly indicates that drug-resistant tumor cells have not expanded. A low plasma-cell labeling index can predict an indolent disease course in asymptomatic patients,[30,31,32] many of whom live longer than 10 years after diagnosis.[6,132]

Clinical Staging of Symptomatic Myeloma

Different centers have defined the extent of myeloma from variations of a schema that considers the degree of anemia, hypercalcemia, and other disease features.[133,134] Based originally on a system devised from direct measurements of total myeloma mass,[135] increasing tumor burden has been associated with shorter survival time.[136] Although the application of different criteria for staging by different centers has prevented a meaningful comparison of treatments between groups, the consistent use within a center or group has allowed more reliable comparisons of new treatments with historical treatments.[137]

Recent studies have improved further the reliability of staging systems. Thus the serum level of beta-2-microglobulin (B_2M), by reflecting both tumor mass and renal function, offers a single measurement that provides as clear a separation of risk groups as any staging system.[138-141] Among 100 previously untreated patients, the authors found progressive shortening of survival time for patients with increasing B_2M levels.[142] Other studies have revealed that the extent of bone lesions is an unreliable index of tumor load, that renal failure occurs much more often with advanced disease, and that the extent, morphology, and DNA/RNA content of marrow plasma cells are important factors. For example, the shorter survival of patients with plasmablastic myeloma may be related to higher proliferative activity,[30] confirmed in several clinical trials to shorten survival independent of tumor mass[34,35,143] or B_2M levels.[144]

Table 6. Diagnosis and Staging

Diagnosis	Differentiating Parameters
MGUS	Normal bones, Hb, Ca, Ig;
	Marrow plasmacytosis <10%
	Labeling index <1%
Indolent myeloma	Few lytic bone lesions without fractures
	Normal Hb, Ca, Ig;
	Marrow plasmacytosis <20%
	Labeling index <1%
Overt myeloma	B_2M <4, 4-6, >6 mg/L
	Durie-Salmon Staging System
	Marrow plasmacytosis <20, 20-40, >40%

In studies evaluating the role of high-dose alkylating agent therapy for VAD-refractory myeloma, the authors observed high-serum LDH levels of >300 U/L prior to or within 2 weeks after therapy in about one half of patients.[114] High LDH conferred a rapidly progressive course with lymphomalike clinical features, in a manner similar to that seen in patients with the blast phase of chronic myelocytic leukemia or with transformed lymphoma. Thus high LDH seems to define a high-grade myeloma as a result of clonal evolution to a more undifferentiated tumor-cell phenotype and/or by progressive expansion of drug-resistant tumor cells.[114] Quantitative studies of tumor-cell LDH content should distinguish these alternatives (Van NT, Epstein J, Barlogie B, unpublished observations, December 1988). Preliminary data in 100 previously untreated patients also showed that high LDH levels occurred more frequently with renal failure and portended a poor prognosis.[142]

Thus there are now a variety of quantitative and tumor cell-derived parameters that correlate with prognosis (Table 7). From a large retrospective analysis of previously untreated patients who received similar chemotherapy, the dominant variables associated with distinct clinical endpoints were determined, eg, (1) absolute resistance with DNA hypodipoloidy and lower remission rates with low plasma-cell RNA index and high B_2M serum levels; (2) shorter remissions with high serum-LDH and low plasma-cell RNA index; and (3) short survival in patients with high serum-LDH and B_2M levels (Table 8). The importance of similar and mainly tumor-intrinsic parameters for both remission induction and long-term prognosis emphasizes the dominant role of marked cytoreduction from initial therapy in each patient's ultimate outcome.

The authors envision that, as in the leukemias and lymphomas, cytogenetic and molecular disease entities will eventually be defined that are associated with unique clinical presentations and prognosis. Meanwhile, relationships among the recognized prognostic factors should be clarified, so that the adverse effects of high LDH,[114] low CALLA expression,[72] high H-ras activity,[111] myelomonocytic phenotype,[74] and high proliferative activity[144] can be traced to few critical biological features to be exploited therapeutically.

Table 7. Prognostic Factors in Myeloma

Endpoint	Adverse Pretreatment Variable	Reference
Response	—DNA hypodiploidy	68
	—Low RNA index	68
	—High tumor mass	68
	—High B_2M	142
	. . . MDR expression	97, 100
Time to relapse	—Low RNA index	142
	—High B_2M	
	—High tumor mass	
	—High LDH	
Survival time	—High B_2M	138-143
	—High tumor mass	136 and many others
	—High LDH	114
	—High labeling index	34, 35, 144
	. . . Low RNA index	68
	. . . DNA hypodiploidy	68
	. . . CALLA-negative	72
	. . . Butyrate esterase-positive	74
	. . . H-ras expressed	111

—, established; . . . , suggested.

OBJECTIVES OF THERAPY

The degree of cytoreduction achieved in most responsive patients rarely exceeds 99%.[145] With most available treatments, disappearance of myeloma protein is evident in only about 10% of those with IgG or IgA peaks, but is evident in about 60% of those with only Bence Jones protein, so that the overall frequency of "complete remission" is about 20%. Rigid criteria for disappearance of abnormal protein should include immunoelectrophoresis and immunofixation techniques. Most centers define a response either as a 50% reduction in myeloma protein concentration[146] or as a 75% reduction in M-protein synthesis[141,148] with disappearance of Bence Jones protein (as in this review). The speed of cytoreduction, calculated from changes in IgG or IgA myeloma protein, varies with different treatments. Thus in responding patients, the median time required for a 50% reduction in IgG or IgA myeloma protein was much shorter with primary VAD (0.4 months) or VCAD-VAD (0.9 months), than with VCAP using bolus vincristine-adriamycin (1.2 months) or standard melphalan-prednisone (2.2 months). High-dose melphalan with total body irradiation achieved the most rapid cytoreduction with protein levels declining in accordance with their expected plasma clearance.

The remission time of responding patients with multiple myeloma has been overlooked as an important endpoint. Few published reports have included this feature in the comparison of different treatments. Now that effective treatment alternatives for remission induction and consolidation are available, remission time assumes more importance in comparing treatments. Disease relapse may be detected early in most patients from rising myeloma protein levels. The median tumor-doubling time for patients with IgG or IgA myeloma protein is about 2 months, with more rapid relapse in those who responded rapidly. In recent years, and especially after VAD, more patients have been recognized with "phenotypic escape."[149] Most commonly this is manifested by increasing bone marrow infiltration with progressive anemia, bone destruction, hypercalcemia, and rising B_2M and LDH levels, despite low or even absent levels of myeloma protein. Transformation to a more aggressive malignancy with rising LDH has been associated with disease in lymph nodes, liver, brain, or blood.[114] Whether such patients are more likely to develop karyotypic or phenotypic changes from those present at diagnosis is not clear. The role of new therapies, such as VAD and/or high-dose melphalan, in causing such changes or allowing them to become manifest because of survival prolongation needs to be assessed.

Table 8. Multivariate Analysis of Prognostic Factors Among 100 Patients Treated at MD Anderson Cancer Center

Endpoint	Adverse Variable In Order of Entry	P
Response	Low RNA (<4)	.001
	High B_2M (>6 mg/L)	.001
Relapse-free survival	LDH (>200 U/L)	.05
	RNA (<4)	.06
Survival	High LDH (>200 U/L)	.001
	High Mass (Durie-Salmon)	.09
	High B_2M (>6 mg/L)	.10

TREATMENT

For the past 25 years, intermittent melphalan-prednisone (MP) has been the therapy of choice for multiple myeloma in community practice. Extensive clinical trials with other drug combinations have not shown a major improvement in disease course.[150,151] Since the details of numerous clinical trials have been summarized elsewhere,[12,13] this review will focus on those studies promising substantial future gain. Since these treatments were developed first for refractory disease, effective salvage regimens will be reviewed prior to discussing their role as initial therapy.

Effective Salvage Regimens

VAD and dexamethasone. Not until the introduction of VAD (combining pulses of high doses of dexamethasone with continuous infusions of vincristine and adriamycin) were there frequent, marked, and durable tumor cytoreductions in patients resistant to standard therapies.[152] Dexamethasone alone was also effective,[85] but VAD induced more frequent remissions in patients with relapsing disease or with primary drug resistance of less than 1 year duration, provided favorable DNA/RNA features of plasma cells were present (Table 9). A longer duration of primary resistance was associated with DNA-hypodiploidy or low plasma-cell RNA index in nearly one half of the patients, providing one biochemical explanation for such resistance. The greater efficacy of VAD than dexamethasone among relapsing patients was attributed to the greater sensitivity of proliferating tumor cells to the cycle-active drugs vincristine and adriamycin. Identical to studies in newly diagnosed patients, a high serum B_2M level (> 6 mg/L) was the dominant adverse feature for survival time.[152]

High-dose melphalan and total body irradiation with bone marrow transplantation. To date, 65 patients with

Table 9. Response to Salvage Treatment Related to Duration of Primary Resistance and to DNA-RNA Features

Treatment	Resistant to	% Responding (No. Treated)	
		Unresponsive for ≤12 months + Favorable DNA/RNA*	Other
DEX	MP	32 (28)	17 (18)
VAD	MP	56 (32)	14 (14)
HDM <90 mg/m²	MP and VAD	67 (12)	0 (8)
HDM 90-140 mg/m²	MP and VAD	47 (15)	53 (15)
HDM 140 mg/m² + TBI	MP and VAD	77 (13)	100 (2)

*Not hypodiploid and RNA index ≥4.

VAD-refractory myeloma have been treated with high dose melphalan (HDM), either alone[153,154] or with total body irradiation[8] (TBI; 850 cGy in five fractions 12 hours apart) and supported with autologous bone marrow. The latter was harvested, whenever possible, during an earlier remission phase and contained less than 30% plasma cells. The rationale for autologous marrow transplantation in this marrow-derived malignancy was based on the predominantly terminal B-cell phenotype with a small proportion of clonogenic tumor cells capable of self-renewal.[42] With the addition of TBI, virtually all patients responded (Table 9), and although the patients were selected more carefully, the median remission and survival times were longer than with the melphalan alone (Table 10).

The previous adverse influence of a long duration of primary drug resistance (>12 months) and of certain DNA/RNA features was no longer observed when high melphalan doses (ie, >90 mg/m²) were employed, indicating both the lack of cross-resistance to VAD and, more importantly, entirely different mechanisms of drug action (Table 9). With better patient performance (Zubrod <2) and normal renal function, early mortality was virtually eliminated. The longest remission and survival times were observed among patients whose LDH levels were less than 300 U/L both prior to and during the first 2 weeks after therapy (Table 10 and Fig 3).[117] The short survival times in patients with higher LDH levels were due to rapid recurrence of a high-grade myeloma with lymphomalike features.

Among patients receiving bone marrow autografts, neither the extent of plasmacytosis nor the tumor-mass kinetics at the time of marrow harvest influenced remission and survival durations.

Since complete remissions with current marrow-ablative regimens occurred rarely and since the phenotype of the myeloma stem cell was unknown, autologous bone marrow purging by immunologic means appears unwarranted. Instead, therapeutic research should focus on the development of regimens that provide even greater cytoreduction. Potential alternatives to marrow autografts include hematopoietic growth factors[155] and/or autologous blood stem cells,[156,157] particularly in patients with extensive marrow plasmacytosis. The combined use of hematopoietic growth factors and autologous stem cells may reduce the duration of severe neutropenia and hence the risk of sepsis with ablative treatments.

Allogeneic bone marrow transplanation. Because of the higher mortality from graft-v-host disease (GVHD) among older patients, the use of allogeneic bone marrow transplantation (BMT) has not been explored systematically. Cyclo-

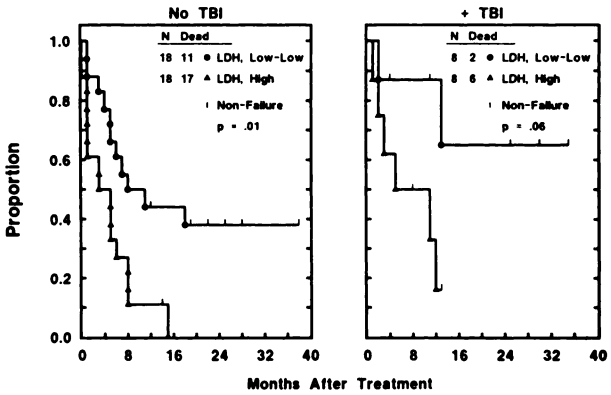

Fig 3. Survival of VAD-refractory myeloma after treatment with HDM with or without TBI (for details, see text). Longest disease control and survival were achieved in patients whose serum LDH levels did not exceed 300 U/L within 2 weeks of therapy, whereas patients with higher levels rarely derived clinical benefit. The prognostic importance of LDH applied whether or not TBI was added.

Table 10. Prognostic Factors with HDM for VAD-Refractory Myeloma

TBI	N	% Response	Median Relapse-Free Survival (months)			Median Survival (months)		
			LDH, Low-Low*	Other†	P	LDH, Low-Low	Other	P
No	36	46	7	3	.04	10	4	.009
Yes	16	80	18	6	.03	35+	8	.07
P		.04	.06	.006		.18	.19	

*LDH, Low-Low: LDH <300 U/L for at least 14 days after treatment.

†Other: LDH ≥300 U/L before or within 14 days after treatment.

phosphamide plus TBI and modifications, with syngeneic or allogeneic BMT, has reduced tumor mass markedly in about one dozen patients, many being treated during a partial remission.[158-161] Monoclonal protein persisted in many patients with progressive or unresponsive myeloma. In a recent European trial, about one half of the patients achieved complete remission, and few patients developed severe GVHD.[162] With the efficacy of anti-CD5 MoAb in glucocorticoid-resistant GVHD, allogeneic marrow transplantation may be better tolerated by older patients.[163] A comparison of autologous with allogeneic BMT (in patients with comparable disease features who received identical cytoreductive therapy) may clarify the possibly adverse effect of autologous tumor-cell reinfusion and the potential gain from a graft-*v*-myeloma effect.

Primary Therapy

VCAD-VAD. Because of its superior activity in melphalan-refractory disease, the VAD regimen has been assessed in previously untreated patients. We have not seen any major gain in survival with VAD or VCAD-VAD that includes oral cyclophosphamide (VCAD) in comparison with preceding regimens, so that no improvement in the frequency of complete remission or survival time has resulted after a 15-year experience with many drug combinations.[142] The lack of survival prolongation with a regimen that was superior for resistant disease is puzzling, particularly since tumor halving times have shortened progressively from a median of 2.2 months with MP to 1.2 months with VCAP and to 0.4 months with VAD. The authors reason that different tumor cells may be affected by the different treatments. VAD with high-dose glucocorticoid may reduce preferentially more differentiated tumor cells so that such manifestations as myeloma protein production, anemia, and hypercalcemia are controlled rapidly. This effect contrasts with standard MP, which may be more effective against myeloma precursors, so that slower but equally sustained tumor control is achieved (Fig 2).

Interferon. Interferon-alpha is an active cytoreductive agent for many patients with low tumor load both at diagnosis and during first remission.[86,164,165] The combination of interferon with VBMCP as initial therapy achieved a higher frequency of complete remission than seen previously.[166] However, interferon rarely benefits patients with resistant or relapsing disease several years after diagnosis. An attractive biological feature of interferon concerns the frequent recovery of normal immunoglobulins in many responding patients, an uncommon event with standard chemotherapy.[164]

HDM ± TBI. The Medical Research Council reported that high-dose intravenous (IV) melphalan produced a 78% response rate (with 27% complete remissions) in 41 newly diagnosed patients with advanced disease.[167,168] A subsequent regimen included initial VAMP (vincristine-adriamycin by continuous infusion plus methylprednisone instead of dexamethasone in VAD) followed, at maximum cytoreduction, by HDM (200 mg/m^2) with autologous remission marrow. Preliminary data indicated a complete remission rate of 30%, with an additional 50% of patients who achieved partial responses; 10% suffered early mortality.[169]

The authors are conducting similar studies in newly diagnosed patients, in which VAD is prescribed for those with intermediate or high tumor mass; responding patients then receive HDM (140 mg/m^2) and TBI (total dose of 850 cGy in five fractions) supported by autologous remission marrow. Only one of five patients treated to date has achieved a complete remission.

Problems and Promises of Therapy

Combinations of alkylating agents with or without adriamycin have not proven superior to standard MP introduced about 25 years ago. During the past 5 years three effective treatments have been discovered: (1) alpha-interferon for low tumor-mass disease; (2) high-dose glucocorticoid therapy[85] with further benefit from continuous-infusion vincristine-adriamycin in proliferating myeloma[152]; (3) HDM with superior antitumor effect from added TBI.[8,129]

The infrequency of complete remissions after marrow-ablative therapies with autologous BMT seems unlikely to be due to tumor-cell reinfusion. This reasoning is based on the slight further reduction of residual myeloma protein in responding patients consolidated during remission and the low number of reinfused plasma cells in proportion to the number persisting in vivo. Furthermore, the infusion of autologous marrow with obvious plasmacytosis has not shortened the duration of remission in patients with refractory myeloma. The "plateau phase" of myeloma during remission may represent a cytokinetic sanctuary this is resistant to high-dose therapy.[170] Alternatively, a BMG-like condition with resistant and long-lived plasma cells may be a common precursor phase of myeloma remaining after successful eradication of the neoplastic tumor-cell population.[129] In addition, one must consider the persistence of causal agents and mechanisms sustaining the monoclonal gammopathy. Thus the continued production of a monoclonal protein after marrow-ablative therapy is not incompatible with durable disease control.

SUMMARY AND CONCLUSIONS

Plasma cell myeloma is a more complex neoplasm than suggested by the relative uniformity of its dominant plasma cells, which represent the terminal stage of normal B-cell differentiation. Phenotypic, molecular, and cellular genetic data favor the presence of a myeloma stem cell early in hematopoietic development so that, as in chronic myelogenous leukemia (CML), a far distance exists between the primordial malignant cell that was the target of malignant transformation and the dominant clinical phenotype. Traces of pre-B, myeloid, and T cells are coexpressed with the mature B-cell phenotype, an occurrence unknown in normal B-cell differentiation.

Analogous to CML, disease progression is marked by disease dedifferentiation, occasionally with cessation of myeloma protein production and development instead of extramedullary lymphomalike features with high LDH or

myelodysplasia/acute myelogenous leukemia (AML) syndromes. The prognostic importance of serum LDH levels even in newly diagnosed myeloma suggests the early presence of tumor cells with "LDH phenotype," which, as a result of drug resistance and proliferative advantage, expand preferentially during disease progression. Further characterization of these cells may provide important clues about the ontogeny of multiple myeloma.

Myeloma cells express many receptors for different biological signals that might be exploitable for therapy with immunotoxins or radioisotopes. Plasma cells and their precursors also produce a variety of cytokines, some of which have putatively autostimulatory functions (eg, IL-1, IL-5, IL-6) and/or are related to disease manifestations (eg, IL-1 and TNF-beta as OAF). The wealth of cellular expression by plasma cells provides clues for understanding the mechanisms of gene activation and the nature of abnormal growth and differentiation.

The accuracy of prognostically relevant staging systems has been refined with the use of new quantitative parameters that reflect tumor mass (ie, serum B_2M levels) and biology. Further studies of cellular and molecular biology (ie, CALLA, H-ras) may reveal those tumor cell features that define clinical entities, response to therapy, and long-term prognosis.

The lack of a major advance in prognosis despite the use of more drugs and more intensive regimens justifies the continued use of standard melphalan-prednisone for patients with a highly favorable prognosis, for the very aged, and for those with a short life expectancy due to other major medical problems.[171] However, a radical departure from standard practice is required to improve the prognosis for younger patients with poor risk features. Especially rational is the application, soon after diagnosis, of three active treatments that lack cross-resistance, namely, alpha-interferon, VAD, and HDM. Exploiting supportive care measures with marrow or blood stem-cell support and/or with hematopoietic growth factors, one should seek a marked tumor reduction with ablative treatments to achieve durable remissions in most patients. As GVHD becomes more preventable and manageable, allogeneic BMT will become more feasible, and with comparative trials, the risk of autologous tumor-cell reinfusion can be assessed. The recent notion of an autocrine growth mechanism, as in acute myeloid leukemia,[172] suggests that future therapy can be designed to interfere more specifically with the abnormal expression of cellular genes, such as H-ras and *c-myc*.

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